(For Laboratory Use Only)
Accuratus Lab Services Project # A19575
Date 11/5/15

ACCURATUS LAB SERVICES

PROTOCOL

Residual Self-Sanitizing Activity of Dried Chemical Residues on Hard Nonporous Surfaces (with exposure and wear activity)

Test Organism:

Staphylococcus aureus (ATCC 6538)

PROTOCOL NUMBER

SRC83102015.RES.1

PREPARED FOR

Byotrol plc
Riverside Works
Collyhurst Road
Manchester M40 7RU
United Kingdom

SPONSOR REPRESENTATIVE

Scientific & Regulatory Consultants, Inc.
201 W. Van Buren Street
Columbia City, IN 46725

PERFORMING LABORATORY

Accuratus Lab Services
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

DATE

October 20, 2015

Revised November 2, 2015

PROPRIETARY INFORMATION

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Residual Self-Sanitizing Activity of Dried Chemical Residues on Hard Nonporous Surfaces (with exposure and wear activity)

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TEST FACILITY: Accuratus Lab Services
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PURPOSE

The purpose of this study is to determine the self-sanitizing activity of antimicrobial products applied to hard, nonporous, inanimate, non-food contact surfaces following exposure and wear activity per EPA O1-1A protocol (Reference 3).

TEST SUBSTANCE CHARACTERIZATION

According to (40 CFR, Part 160, Subpart F [160.105]) test substance characterization as to identity, strength, purity, solubility and composition, as applicable, shall be documented before its use in this study. The stability of the test substance shall be determined prior to or concurrently with this study. Pertinent information, which may affect the outcome of this study, shall be communicated in writing to the Study Director upon sample submission to Accuratus Lab Services. Accuratus Lab Services will append Sponsor-provided Certificates of Analysis (C of A) to this study report, if requested and supplied. Characterization and stability studies not performed following GLP regulations will be noted in the Good Laboratory Practice compliance statement.

SCHEDULING AND DISCLAIMER OF WARRANTY

Experimental start dates are generally scheduled on a first-come/first-serve basis once Accuratus Lab Services receives the Sponsor approved/completed protocol, signed fee schedule and corresponding test substance(s). Based on all required materials being received at this time, the proposed experimental start date is November 9, 2015. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of December 9, 2015. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at Accuratus Lab Services.

A "case-by-case" approach is generally taken by the regulatory authorities and cannot be over-emphasized when considering a testing regimen. While this protocol is based upon our experience in the field of germicidal testing, and the current EPA guidelines, each product presents a different set of issues to the regulatory authorities. We recommend that you consult with the appropriate agency before finalizing your testing regimen, as Accuratus Lab Services cannot guarantee acceptance of this protocol by the regulating authorities. If a test must be repeated, or a portion of it, due to failure by Accuratus Lab Services to adhere to specified procedures, it will be repeated free of charge. If a test must be repeated, or a portion of it, due to failure of internal controls, it will be repeated free of charge. "Methods Development" fees shall be assessed, however, if the test substance and/or test system require modifications due to complexity and difficulty of testing. If the Sponsor requests a repeat test, they will be charged for an additional test. Neither the name of Accuratus Lab Services nor any of its employees are to be used in advertising or other promotion without written consent from Accuratus Lab Services. The Sponsor is responsible for any rejection of the final report by the regulating agencies concerning report format, pagination, etc. To prevent rejection, Sponsor should carefully review the Accuratus Lab Services final report and notify Accuratus Lab Services of any perceived deficiencies in these areas before submission of the report to the regulatory agency. Accuratus Lab Services will make reasonable changes deemed necessary by the Sponsor, without altering the technical data.

JUSTIFICATION FOR SELECTION OF THE TEST SYSTEM

The U.S. Environmental Protection Agency requires that a specific claim for a test substance intended for use on hard surfaces be supported by appropriate scientific data demonstrating the efficacy of the test substance against the claimed organism. This is accomplished by treating a test surface with the test substance under conditions, which simulate as closely as possible, in the laboratory, the actual conditions under which the substance is designed to be used. For products intended for use on hard surfaces (dry, inanimate environmental surfaces), a carrier method is used in the generation of the supporting data. The experimental design in this protocol meets these requirements. The test system to be used in this study will follow the methods described in the Protocol for Residual Self-Sanitizing Activity of Dried Chemical Residues on Hard, Non-porous Surfaces (Reference 3).

TEST PRINCIPLE

This protocol describes the microorganisms, equipment, data collection and procedures used for evaluating a residual sanitizer for non-food contact surfaces. This method includes a regimen by which each treated surface undergoes specific wear exposures to demonstrate residual efficacy of the test product. Appropriate numbers control, culture purity, sterility, initial suspension and neutralization confirmation controls will be performed. The current version of Standard Operating Procedure CGT-0051 reflects the methods which shall be used in this study.

TEST METHOD

Test Organism	ATCC #	Growth Medium	Incubation Parameters
Staphylococcus aureus	6538	Nutrient Broth	35-37°C, aerobic

The test organism to be used in this study was obtained from the American Type Culture Collection (ATCC), Manassas, VA.

Carriers

Non-frosted glass (1" x 1") surfaces will be used in testing. Clean each carrier by dipping in ethyl alcohol and rinsing thoroughly in deionized water. After cleaning, decontaminate the surfaces by autoclave sterilization. (Alternatively, the carriers may be decontaminated by dipping in absolute ethanol and aseptically allowing the carriers to dry in a bio-safety hood.) Transfer the carriers aseptically to Petri dishes lined with 2 pieces of Whatman #2 filter paper.

Preparation of the Test Organism

From a stock slant, an initial 20 x 150 mm tube (10 mL) of culture broth will be inoculated and incubated at 35-37°C for 18-24 hours. This culture is termed the "initial broth suspension." From this initial broth suspension, a minimum of three daily transfers using 1 loopful (10 µL) of culture into 10 mL of culture media will be performed on consecutive days prior to use in testing procedure.

a) For the **initial inoculation culture**, vortex-mix a 48-54 hour culture for 3-4 seconds and let stand for 15±1 minutes. Using the upper 2/3rds of inoculum, serially dilute the culture by adding 0.1 mL of culture to 9.9 mL of sterile deionized water. Repeat this serial dilution a second time **yielding a total of two 1:100 dilutions**. The concentration of the final (diluted) initial inoculation culture(s) will be determined by serial dilution and standard pour plating technique (initial suspension control). An organic soil load may be added to the diluted culture per Sponsor's request. The final culture will be mixed and allowed to stand at least 15±1 minutes prior to use.

b) For the **reinoculation culture**, vortex-mix an 18-24 hour culture for 3-4 seconds and let stand for 15±1 minutes. Using the upper 2/3rds of inoculum, serially dilute the culture by adding 0.1 mL of culture to 9.9 mL of sterile deionized water. Repeat this serial dilution a second time **yielding a total of two 1:100 dilutions**. Finally, dilute the culture 1:2 by combining 5.0 mL of culture with 5.0 mL of sterile deionized water (or equivalent dilution). The concentration of the final (diluted) 18-24 hour reinoculation culture(s) will be determined by serial dilution and standard pour plating technique (initial suspension control). An organic soil load may be added to the diluted culture per Sponsor's request. The final culture will be mixed and allowed to stand at least 15±1 minutes prior to use. No culture with organic soil load will be allowed to stand >8 hours prior to use.

c) For the **sanitizer test culture**, vortex-mix an 18-24 hour culture (See modification 5) for 3-4 seconds and let stand for 15±1 minutes. Remove the upper 2/3rds of inoculum by aspiration for inoculation. The concentration of this undiluted sanitizer test culture will be determined by serial dilution and standard pour plating technique (initial suspension control). An organic soil load may be added per Sponsor's request. The final culture will be mixed and allowed to stand at least 15±1 minutes prior to use.

Initial Inoculation Procedure
Using the prepared initial inoculation culture, apply a 10 µL aliquot to each test and numbers control carrier spreading the inoculum with a **bent needle (hook)** to within approximately 1/8th inch from the edge of the carrier. Dry the carriers, with the Petri dish lids ajar, at 35-37°C for 30-35 minutes, or until visibly dry.

Preparation of Test Substance
The test substance(s) to be assayed will be used as directed by the Sponsor. If a dilution of the test substance is requested by the Sponsor, the diluted test substance(s) shall be used within three hours of preparation.

Application of the Test and Control Substance
Apply the test substance to each inoculated, dried test carrier as directed by the Sponsor. Allow the surfaces to dry on a level surface at ambient temperature (approximately 15-25°C) and 45-55% relative humidity for at least 3 hours, or until completely dry. Overnight drying may be necessary. A humidity chamber may be used to achieve these conditions. The Petri dish lids will be left ajar during the drying procedure.

Similarly, apply a sterile solution of 0.01% Triton X-100 solution to each inoculated, dried numbers control carrier. For wipe applications, the control carriers may be treated by misting the carriers with 0.01% Triton X-100. Allow the control carriers to dry as described for the test carriers.

Wear Procedure

Calibration of the abrasion tester
Set the abrasion tester to the number of cycle passes to be used in the actual wear procedure. One cycle pass initiates the abrasion boat to pass over the carrier and return back over the carrier. *(Note: The number of cycle passes is 1 unless otherwise noted.)* Set the speed of the abrasion tester to approximately 2.25 to 2.5 targeting a total surface contact time of approximately 4-5 seconds for one complete pass. (One complete pass represents the time each abrasion boat is in contact with the carrier as it passes over and returns back over the carrier.) Verify, using a calibrated stopwatch, that the contact time for one complete pass is equal to 4 to 5 seconds. Adjust the speed as necessary. Perform this calibration procedure each day wear cycles are performed.

Wear procedure
Inoculated, treated and dried test and numbers control carriers will undergo a wear and reinoculation regimen, which will take place over ≥ 24 hours at ambient temperature and humidity conditions. (Two carriers will undergo the wearing procedure simultaneously, per abrasion boat.) Each abrasion boat apparatus will be assembled with sufficient weights, a foam liner and a sterile cotton liner such that the actual weight of the assembled boat is equal to 1084 ± 0.2g. The actual weight of each abrasion boat assembly will be recorded each time it is assembled and used.

In between wear cycle sets, each abrasion boat apparatus will be disassembled and the cotton liner will be replaced with a fresh, sterile cotton liner. The foam liner will be replaced as needed and between organisms. Additionally, each abrasion tester will be decontaminated with absolute ethanol in between cycle sets allowing the alcohol to completely evaporate before re-use.

Alternating dry and wet cycles will be performed. Wet wear cycles will be performed by wetting the cotton liner attached to the weight boat assembly with sterile deionized water, using a Preval sprayer (or equivalent). This can be achieved by misting the liner from a distance of approximately 75±1 cm for not more than one second. Immediately after wetting, each moistened abrasion boat will be attached to the abrasion tester and will be used.

Reinoculation procedure
After an entire wear cycle is complete (i.e. all test and control carriers have undergone the wear procedure), each test and numbers control carrier will be reinoculated. Reinoculation, as applicable, must occur ≥ 15 minutes after the wear procedure was performed for the given carrier. Using the prepared reinoculation culture inoculum, apply a 10 µL aliquot to each carrier spreading the inoculum with a bent needle (hook) to within approximately 1/8th inch from the edge of the carrier. Allow ≥ 15 minute between wear cycles 6-12.

Dry the reinoculated carriers for ≥ 30 minutes at ambient temperature prior to initiating the next wear cycle or the sanitizer test. Lids may be left slightly ajar to aid in drying.

Actual ambient conditions will be periodically measured during the wear and reinoculation procedure. A continuous monitoring device such as a chart recorder may be used. While it is desired to achieve ambient conditions with 45-55% relative humidity, this humidity range may fluctuate seasonally and therefore cannot be guaranteed.

Refer to the following sample wear and reinoculation procedure used for 12 wear cycles, alternating wet and dry cycles with 5 reinoculations. This is only an example; alternative schedules/procedures may be followed where appropriate maintaining protocol adherence.

Day	Procedure
1	Initial inoculation / drying
	Test / Control Substance application and drying
	Controls: Initial suspension(s), purity, sterility controls, neutralization confirmation control etc.
2-3	Wear cycle #1 (dry)
	Reinoculation #1 / drying
	Wear cycle #2 (wet)
	Reinoculation #2 / drying
	Wear cycle #3 (dry)
	Reinoculation #3 / drying
	Wear cycle #4 (wet)
	Reinoculation #4 / drying
	Wear cycle #5 (dry)
	Reinoculation #5 / drying
	Wear cycle #6 (wet)
	Controls: Initial suspension(s), purity, soil sterility (if applicable) etc.
	Wear cycle #7 (dry)
	Wear cycle #8 (wet)
	Wear cycle #9 (dry)
	Wear cycle #10 (wet)
	Wear cycle #11 (dry)
	Wear cycle #12 (wet)
	Controls: Initial suspension(s), purity, soil sterility (if applicable)
3	Sanitizer test / numbers control evaluation
	Controls: Initial suspension(s), purity, soil sterility (if applicable), additional media sterility (if applicable) etc.

Sanitizer Test
At least 15 minutes after the final wear cycle (and at least 24 hours after test substance application), the sanitizer test will be initiated. Using the prepared sanitizer test culture, inoculate each test and numbers control carrier with 10 µL of culture spreading the inoculum with a bent needle (hook) to within approximately 1/8th inch from the edge of the carrier. Apply the culture at staggered intervals using a calibrated timer. Allow the carriers to expose at ambient conditions for the exposure period specified by the Sponsor. Exposure begins for each carrier as it is inoculated.

Once the exposure period has been achieved, begin subculturing each test and numbers control carrier (at identical staggered intervals) into **30 mL** of neutralizer broth using sterile forceps (representing a 10^0 dilution). Continue until all test and numbers control carriers have been subcultured.

Following subculturing, sonicate each subculture for approximately 20±2 seconds. Mix each sonicated subculture on an orbital shaker set to approximately 250 RPM for 3-4 minutes. Within 30 minutes of neutralization, prepare ten-fold serial dilutions using a sterile diluent.

For the test subcultures, pour-plate 1.0 mL aliquots of 10^0 through 10^{-3} in duplicate using an appropriate subculture agar medium (e.g. TSA agar). If neutralization is a concern, the aliquots may be transferred to filter units pre-wetted with at least 10 mL of sterile saline, evacuated and rinsed with ≥ 50 mL of sterile saline. The filters are then aseptically plated onto appropriate agar.

For the numbers control carriers, pour-plate 1.0 mL aliquots of 10^{-1} through 10^{-4} in duplicate using an appropriate subculture agar medium (e.g. TSA agar).

Incubation and Observation
Incubate plates and controls at 35-37°C for **48-54 hours**. If necessary, the subcultures may be refrigerated at 2-8°C for up to three days prior to examination.

Following incubation, the subcultures will be visually examined for growth. If possible, count plates containing between 30 and 300 CFU.

Representative test subcultures will be stained and/or biochemically assayed to confirm or rule out the presence of the test organism. If applicable, growth from a minimum of 2 test subcultures will be assayed.

STUDY CONTROLS

Numbers Control
The numbers control procedure will be performed as outlined throughout this test protocol. The acceptance criterion for this study control is a minimum geometric mean of 3.0×10^4 CFU/carrier which is required to show a 99.9% reduction when 30 mL of neutralizer is used. This control can also be used to demonstrate culture viability.

Purity Control
Each test organism culture used **on each day of testing** will be streaked to an appropriate agar for isolation and incubated as in the test. The acceptance criterion for this control is a pure culture demonstrating colony morphology typical of the test organism.

Organic Soil Load Sterility Control
If applicable, 1.0 mL of the soil used will be added to a tube of Fluid Thioglycollate Medium and will be incubated as in the test. This control will be performed for each container/lot of soil used and **will be performed each day the soil is used.** The acceptance criterion for this study control is no growth.

Carrier Sterility Control
A representative uninoculated carrier will be added to neutralizer broth. A 1.0 mL aliquot will be plated using appropriate agar. The plate will be incubated as in the test and will be examined for growth. The acceptance criterion for this study control is a lack of growth.

Neutralizer Sterility Control
A 1.0 mL aliquot of untreated neutralizer (for each lot used) will be plated, incubated as in the test and examined for growth. The acceptance criterion for this study control is a lack of growth.

Initial Suspension Control
Each prepared test organism suspension used (i.e. diluted initial inoculation cultures, diluted reinoculation cultures and sanitizer test cultures) will be serially diluted and pour-plated following standard microbiological technique. This will be performed each day each culture is used and will be incubated as in the test. This study control has no acceptance criteria and is used for informational purposes only.

Neutralization Confirmation (NC) Control
A neutralization confirmation control will be performed to ensure adequate neutralization. This control may be performed prior to testing or concurrent with testing. If multiple concentrations of the test substance are utilized in testing, only the most concentrated test substance needs to be evaluated in this control.

Sterile test carriers will be treated with the test substance as in the test and will be allowed to air dry. Similarly, sterile test carriers will be treated with 0.01% Triton X-100 to be used as a numbers control. For wipe applications, the control carriers may be treated by misting the carriers with 0.01% Triton X-100.

Following drying, the treated test and numbers control carriers will be transferred to 30 mL of neutralizer as in the test using staggered intervals. Challenge each subculture with 1.0 mL of a low level of test culture diluted to target <200 CFU per mL of neutralizer. (Multiple organism dilutions may be prepared.). The vessels will be mixed and allowed to stand for 5±1 minutes. Following standing, duplicate 1.0 mL aliquots will be removed from each vessel and pour-plated (or filter plated as in the test). The acceptance criterion for this study control is growth within 70% of the numbers control.

PROCEDURE FOR IDENTIFICATION OF THE TEST SYSTEM

Accuratus Lab Services maintains Standard Operating Procedures (SOPs) relative to efficacy testing studies. Efficacy testing is performed in strict adherence to these SOPs which have been constructed to cover all aspects of the work including, but not limited to, receipt, log-in, and tracking of biological reagents including test organism strains for purposes of identification, receipt and use of chemical reagents. These procedures are designed to document each step of efficacy testing studies. Appropriate references to medium, batch number, etc. are documented in the raw data collected during the course of each study.

Additionally, each efficacy test is assigned a unique Project Number when the protocol for the study is initiated by the Study Director. This number is used for identification of the test subcultures, etc. during the course of the test. Test subcultures are also labeled with reference to the test organism, experimental start date, and test product. Microscopic and/or macroscopic evaluations of positive subcultures are performed in order to confirm the identity of the test organism. These measures are designed to document the identity of the test system.

METHOD FOR CONTROL OF BIAS: NA

STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria
To be defined as a residual sanitizer, the test substance must demonstrate a minimum test organism reduction of 99.9% following wear activity and exposure.

Control Acceptance Criteria
The study controls must perform according to the criteria detailed in the study controls description section. If any of the control acceptance criteria are not met, the test may be repeated under the current protocol number.

REPORT

The report will include, but not be limited to, identification of the sample, date received, initiation and completion dates, identification of the bacterial strains used, description of media and reagents, description of the methods employed, tabulated results and conclusion as it relates to the purpose of the test, and all other items required by 40 CFR Part 160.185.

PROTOCOL CHANGES

If it becomes necessary to make changes in the approved protocol, the revision and reasons for changes will be documented, reported to the Sponsor and will become a part of the permanent file for that study. Similarly, the Sponsor will be notified as soon as possible whenever an event occurs that may have an effect on the validity of the study.

Standard operating procedures used in this study will be the correct effective revision at the time of the work. Any minor changes to SOPs (for this study) or methods used will be documented in the raw data and approved by the Study Director.

TEST SUBSTANCE RETENTION

It is the responsibility of the Sponsor to retain a sample of the test substance. All unused test substance will be discarded following study completion unless otherwise indicated by Sponsor.

RECORD RETENTION

Study Specific Documents

All of the original raw data developed exclusively for this study shall be archived at Accuratus Lab Services for a minimum of five years for GLP studies or a minimum of six months for all other studies following the study completion date. After this time, the Sponsor (or the Sponsor Representative, if applicable) will be contacted to determine the final disposition. These original data include, but are not limited to, the following:

1. All handwritten raw data for control and test substances including, but not limited to, notebooks, data forms and calculations.
2. Any protocol amendments/deviation notifications.
3. All measured data used in formulating the final report.
4. Memoranda, specifications, and other study specific correspondence relating to interpretation and evaluation of data, other than those documents contained in the final study report.
5. Original signed protocol.
6. Certified copy of final study report.
7. Study-specific SOP deviations made during the study.

Facility Specific Documents

The following records shall also be archived at Accuratus Lab Services. These documents include, but are not limited to, the following:

1. SOPs which pertain to the study conducted.
2. Non study-specific SOP deviations made during the course of this study which may affect the results obtained during this study.
3. Methods which were used or referenced in the study conducted.
4. QA reports for each QA inspection with comments.
5. Facility Records: Temperature Logs (ambient, incubator, etc.), Instrument Logs, Calibration and Maintenance Records.
6. Current curriculum vitae, training records, and job descriptions for all personnel involved in the study.

REFERENCES

1. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Uses of Antimicrobial Agents, September 4, 2012.

2. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2300: Sanitizers for Use on Hard Surfaces- Efficacy Data Recommendations, September 4, 2012.

3. Protocol for Residual Self-Sanitizing Activity of Dried Chemical Residues on Hard, Non-porous Surfaces. Protocol number 01-1A. *www.epa.gov/oppad001/cloroxpcol_final.pdf*

DATA ANALYSIS

Calculations

CFU/mL for initial suspension = $\frac{\text{(average CFU/plate at the dilution) x (dilution factor)}}{\text{(volume plated in mL)}}$

Number of Organisms Surviving per Carrier

CFU/carrier = $\frac{\text{(average CFU) x (dilution factor) x (volume neutralized solution in mL)}}{\text{(volume plated or filtered in mL)}}$

The carrier population control will be calculated using data from the most appropriate dilution.

Geometric Mean of Number of Organisms Surviving on Test or Control Carriers

Geometric Mean = Antilog of $\frac{\text{Log}_{10}X_1 + \text{Log}_{10}X_2 + \text{Log}_{10}X_N}{N}$

Where: X equals CFU/carrier
N equals number of carriers

Percent Reduction

% reduction = [(a - b) / a] x 100

where:
a = geometric mean of the number of organisms surviving on the numbers control carriers.
b = geometric mean of the number of organisms surviving on the test carriers.

Neutralization Confirmation Control % Recovery = $\frac{\text{(Average CFU for Treated Test carrier) x 100}}{\text{(Average CFU for Numbers Control)}}$

Statistical Analysis
None used.

STUDY INFORMATION

(All blank sections are completed by the Sponsor or Sponsor Representative as linked to their signature, unless otherwise noted.)

Test Substance (Name and Batch Number - exactly as it should appear on final report):
Valhalla, Lots: RSH018/38, RSH018/39 and RSH018/40
Testing at the lower certified limit (LCL) is required for registration, no aged batch is necessary.

Product Description:
☑ Quaternary ammonia ☐ Peracetic acid ☐ Iodophor ☐ Peroxide
☐ Sodium hypochlorite ☑ Other PHMB

Approximate Test Substance Active Concentration (upon submission to Accuratus Lab Services):
<0.485% PHMB; <0.485% Quat
(This value is used for neutralization planning only. This value is not intended to represent characterization values.)

Neutralization/Subculture Broth: ☑ DE Broth + 0.14% Lecithin + 1.0% Tween 80 (30 mL volume)
☐ Accuratus Lab Services' Discretion. By checking, the Sponsor authorizes Accuratus Lab Services, at their discretion, to perform neutralization confirmation assays at the Sponsor's expense prior to testing to determine the most appropriate neutralizer. (See Fee Schedule).

Storage Conditions
☑ Room Temperature
☐ 2-8°C
☐ Other:

Hazards
☐ None known: Use Standard Precautions
☑ Material Safety Data Sheet, Attached for each product
☐ As Follows:

Product Preparation
☑ No dilution required, Use as received (RTU)
☐ *Dilution(s) to be tested:
_____ defined as _____ + _____
(example: 1 oz/gallon) (amount of test substance) (amount of diluent)
☐ Deionized Water (Filter or Autoclave Sterilized)
☐ Tap Water (Filter or Autoclave Sterilized)
☐ AOAC Synthetic Hard Water: _____PPM
☐ Other _____
Note: An equivalent dilution may be made unless otherwise requested by the Sponsor.

Test Organism: ☑ *Staphylococcus aureus* (ATCC 6538)

Carrier Number: 4 test carriers & 4 control carriers **Carrier Surface Type:** ☑ Glass

Spraying Time or # of Sprays: Prime sprayer thoroughly and spray 3 times or until thoroughly wet, at a ~45° angle

Approximate Spraying Distance: 6-8 inches

Exposure Temperature: Ambient **Hold time:** At least 3 hours or until dry

Number of wear cycles: 12 **Number of Reinoculations** ☑ 5 ☐ _____

Number of wear cycle passes: 1 (1 cycle will pass over the carrier twice – over and back.)

Exposure Time: 4.5 Minutes ±5 seconds (Time period following final carrier inoculation, prior to subculture)

Organic Soil Load:

- ☑ Minimum 5% Organic Soil Load (Heat Inactivated Fetal Bovine Serum)
- ☐ No Organic Soil Load Required
- ☐ Other _____

TEST SUBSTANCE SHIPMENT STATUS

(This section is for informational purposes only.)

- ☑ Test Substance is already present at Accuratus Lab Services.
- ☐ Test Substance has been or will be shipped to Accuratus Lab Services.
  Date of expected receipt at Accuratus Lab Services: 9/10/15
- ☐ Test Substance to be hand-delivered (must arrive by noon at least one day prior to testing or other arrangements made with the Study director)

COMPLIANCE

Study to be performed under EPA Good Laboratory Practice regulations (40 CFR Part 160) and in accordance to standard operating procedures.

- ☑ Yes
- ☐ No (Non-GLP or Development Study)

PROTOCOL MODIFICATIONS

- ☐ Approved without modification
- ☑ Approved with modification
  1) Fill the spray containers provided with approximately 500 mL of solution. This spray container may be used across multiple studies until the volume is no longer sufficient to get through a given study. The container can be discarded at that time and a new spray container filled. The filled spray container should be stored at room temperature.
  2) Standardize the *S. aureus* sanitizer culture to achieve a target of 5.8-6.38 $\log_{10}$/carrier by making a 1:2 dilution in **growth medium**. These levels are based on ASTM E1153 to standardize testing.
  3) After application of the test or control substance: Maintain the carriers at ambient temperature and 45-55% relative humidity throughout testing, with the exception of during the wear cycles.
  4) Wear cycles will be conducted using **two abrasion boats** simultaneously.
  5) For the Sanitizer culture, target use of an 18-19 hour culture and use in testing within ~1 hour of removal from incubator to standardize testing. Multiple transfers may be performed the day prior where needed (for example, 1 pm and 3 pm).

PROTOCOL ATTACHMENTS

Supplemental Information Form Attached - ☐ Yes ☑ No

TEST SUBSTANCE CHARACTERIZATION & STABILITY TESTING
[Verification required per 40 CFR Part 160 Subpart B (160.31(d))].

☐ Characterization/Stability testing is not required (For Non-GLP or Development testing only)

OR

Physical and Chemical Characterization (Identity, purity, strength, solubility, as applicable) of the test lots

☑ **Physical & Chemical Characterization has been or will be completed prior to efficacy testing.**

GLP compliance status of physical & chemical characterization testing:
☑ Testing was or will be performed following 40 CFR Part 160 GLP regulations
☐ Characterization has not been or will not be performed following GLP regulations

Check and complete the following that apply:
☑ A Certificate of Analysis (C of A) has been or will be provided for each lot of test substance to be appended to the report.
☑ Testing has been or will be conducted at Accuratus Lab Services under protocol or study #:
SRC83082815.CHR (A19285)
☐ Test has been or will be conducted by another facility under protocol or study #:
_____

☐ **Physical & Chemical Characterization was not or will not be performed prior to efficacy testing.**

Stability Testing of the formulation

☑ **Stability testing has been or will be completed prior to or concurrent with efficacy testing.**

GLP compliance status of stability testing:
(GLP compliance is required by 40 CFR Part 160)
☑ Testing was or will be performed following 40 CFR Part 160 GLP regulations
☐ Stability testing has not been or will not be performed following GLP regulations

Check and complete the following that apply:
☐ Testing has been or will be conducted at Accuratus Lab Services under protocol or study #:
_____
☐ Test has been or will be conducted by another facility under protocol or study #:
_____

☐ **Stability testing was not or will not be performed prior to or concurrent with efficacy testing.**

If test substance characterization or stability testing information is not provided or is not performed following GLP regulations, this will be indicated in the GLP compliance statement of the final report.

APPROVAL SIGNATURES

SPONSOR:

NAME: Ms. Rhonda Jones TITLE: Agent

SIGNATURE: [signature] DATE: 11-3-15

PHONE: (260) 244 - 6270 FAX: 260-244-6270 EMAIL: rjones@srcconsultants.com

For confidentiality purposes, study information will be released only to the sponsor/representative signing the protocol (above) unless other individuals are specifically authorized in writing to receive study information.

Other individuals authorized to receive information regarding this study: ☐ See Attached

Stephanie Burke, Trevor Francis and SRC Staff

Accuratus Lab Services:

NAME: Matthew Sathe
Study Director

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Study Director DATE: 11-5-15